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REVIEW ARTICLE

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Sac-like pouches in *Blastocystis* from the house lizard *Cosymbotus platyurus*

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The mode of transmission of the intestinal protozoan parasite *Blastocystis hominis* continues to remain in doubt. The suggestion that the intimate association between humans and animals (Doyle et al. 1990) could facilitate transmission led us to investigate the prevalence of *Blastocystis* in animals and insects in the domestic environment. The present paper reports the occurrence of the parasite in common household lizards and cockroaches. Whereas the latter has been documented elsewhere (Zaman et al. 1993), the biological features of the parasite in the former are under investigation.

A total of 30 house lizards (*Cosymbotus platyurus*), cockroaches (*Periplaneta americana*), and houseflies (*Musca domestica*), respectively, were caught in dwellings in the city. All house lizards and cockroaches caught were anesthetized. The stomach walls were cut open and the intestinal contents were cultured in bijou bottles containing Jones' medium (Jones 1946) at room temperature for 48 h. Houseflies were crushed and placed in bijou bottles containing the same medium. The contents of the culture bottle were examined at 48 h after incubation at room temperature with a microscope at × 400 magnification. Specimens were also studied using transmission electron microscopy (TEM, Philips 400T) as previously described (Teow et al. 1991).

In all, 7% of the captured house lizards and 10% of the cockroaches caught were positive for *Blastocystis*. The parasites found in cultures from the intestinal contents of house lizards were rounded, numerous in number, and of varying size and showed the typical peripheral nuclei characteristic of *Blastocystis*. Most of the parasites seen were granular forms (Fig. 1). The granules were prominent and refractile. Cyst-like forms of *Blastocystis* were seen, with one of the parasites being enclosed within an outer membrane (Fig. 2) and another displaying a highly refractile, thickened cytoplasm (Fig. 3).

TEM studies revealed that many of the parasites had stages that were multivacuolar in nature (Fig. 4). Some of them had rounded mitochondria that showed prominent cristae (Fig. 4). One of the parasites contained three large vacuoles (Fig. 5). These vacuoles were bordered by a distinct membrane and had the appearance of sac-like pouches enclosing rounded granules. One of the vacuoles showed a rupture of the membrane, releasing these granules to the outer central body of the parasite. At higher magnification it was possible to see that theses granules were the progeny of the parasite, showing prominent nuclei and central bodies (Fig. 6).

Zaman et al. (1993) reported that 80% of the cockroaches caught from sewage tanks in their study were positive for *Blastocystis*. The low incidence found in the present study can be attributed to the observation that the cockroaches were caught from dwellings, not from sewage tanks. None of the houseflies caught was positive for the parasite.

Blastocystis seems to be common in reptiles. Teow et al. (1992) found *Blastocystis* in 8 of 23 (28.6%) species of reptiles housed at the Singapore Zoological Gardens. *Blastocystis* have been isolated from sea snakes (*Lapemis hardwickii*), tortoises (*Geochelone elephantopus*), the crocodile (*Crocodylus porosus*), and the iguana lizard (*Cyclura cornuta*; Teow et al. 1991). The vacuolar and

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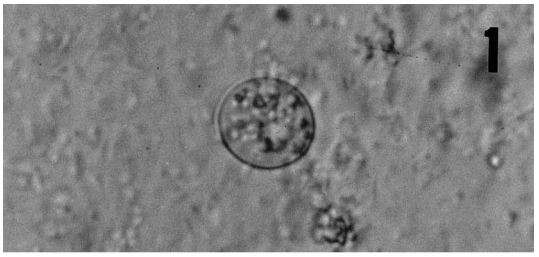
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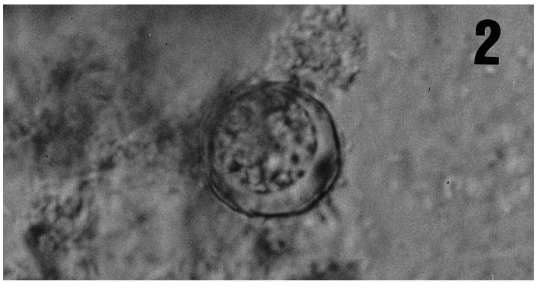
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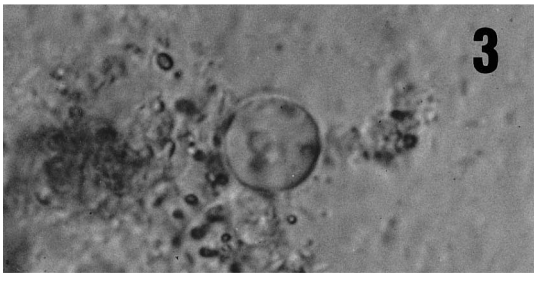
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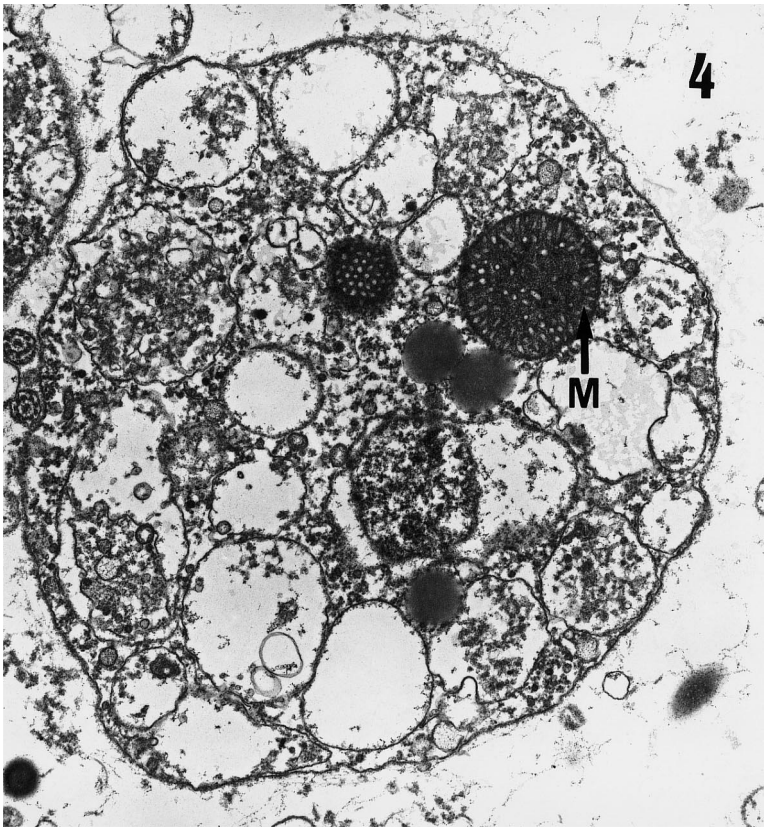
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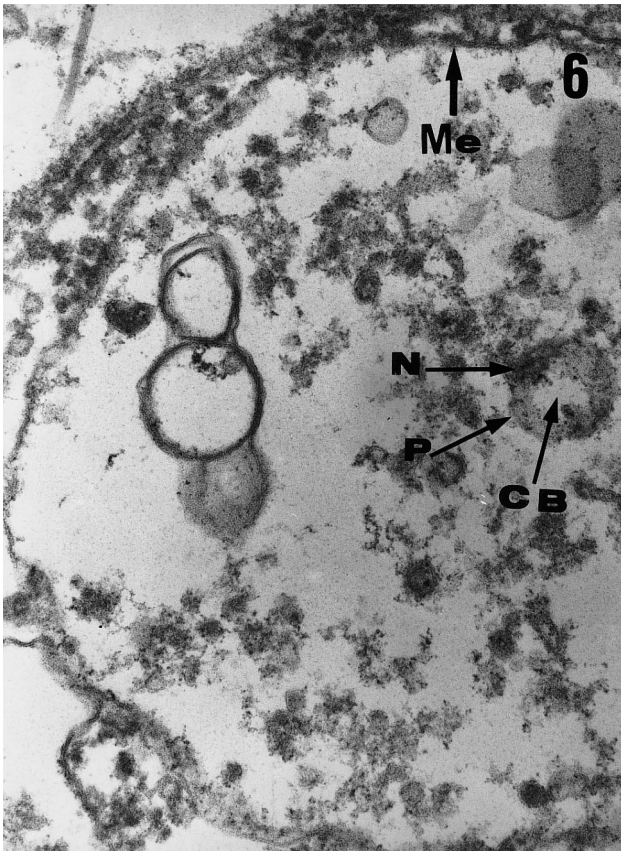
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Fig. 1 Granular forms of *Blastocystis* from cultures of the intestinal contents of house lizards. Note the prominent granules, which are very refractile. × 400. **Fig. 2** Cyst-like forms of *Blastocystis*, with one of the parasites being enclosed within an outer membrane. × 400. **Fig. 3** Cyst-like forms of *Blastocystis* with a highly refractile, thickened cytoplasm. × 400. **Figs. 4–6** Transmission electron micrographs of *Blastocystis*. **Fig. 4** Multivacoular forms. Note the mitochondria (*M*) with prominent cristae × 100,000. **Fig. 5** Parasite containing three large vacuoles bordered by a distinct membrane (*Me*). Note the rupture of one of the vacuoles, which is releasing progeny (*P*). × 550,000. **Fig. 6** Higher magnification of one of the vacuoles. Note the distinct membrane (*Me*)-bound vacuole giving the appearance of a sac-like pouch. Also note the progeny (*P*) with a distinct nucleus (*N*) and central body (*CB*) within the vacuole. × 620,000

granular forms of these isolates were similar to those of *B. hominis*.

In the present study, most of the parasites were granular forms ranging in size from 10 to 20 μm. Similar cyst-like stages with an outer surrounding membrane have been reported in cockroaches (Zaman et al. 1993). Cystic stages with a thickened, refractile cytoplasm similar to that seen in the present study have been reported elsewhere (Suresh et al. 1994). The prominent granules seen in the present study were shown by TEM to be reproductive granules. Reproductive granules were seen in the cystic stages of *B. hominis* that were similar to those demonstrated in previous studies using TEM (Suresh et al. 1994) and scanning electron microscopy (Suresh et al. 1994a).

In the present TEM study we noticed that these granules developed into progeny within sac-like pouches. Boreham and Stenzel (1993) believed that the intestinal forms observed in vivo were avacuolar and that these forms gave rise to multivacuolar forms seen in fresh feces. However, in the proposed life cycle of *Blastocystis* (Singh et al. 1995) the multi-vacuolar stage has been shown to arise from the vacuolar stage. In the present study it was difficult to determine whether these sac-like pouches arose from the coalescing of the smaller vacuoles seen in the multivacuolar stages or from the vacuolar stage itself. The formation of rounded, sac-like pouches and conversion of the potentially reproductive granules into progeny requires energy, which may explain why a rounded mitochondria possessing prominent cristae was seen.

The present observation implies that the formation of multiple vacuoles may not be an accidental occurrence in nature but may rather be a deliberate means of facilitating reproduction in the parasite. It is highly probable that the development of progeny takes place within the vacuole. The vacuole then ruptures, as is implied by the break seen in the membrane, and the developed progeny are subsequently released into the central body of the mother *Blastocysis*. To the best of our knowledge, such reproductive mechanisms have not been reported in other protozoans. Attempts are presently being made to axenize the isolates for speciation studies.

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